3541-Pos Board B402
Active Brownian Dynamics Applied to a Molecular Motor System
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An active Brownian particle in our generalized energy-depot model is able to convert the internal energy into mechanical energy via a non-linear conversion mechanism. In this work we analyze the model assuming that the energy conversion rate function consists of linear and quadric terms of the particle's velocity. We develop a simple model which describes the motion of diverse molecular motors and provide a basic idea on the physical and biological mechanisms behind the dynamics of molecular motors. The active motion driven by a stochastic energy supply is investigated under the influences of ratchet potential, external load, thermal noise, and ATP concentration by adopting the experimentally well-known realistic parameters of kinesin-1.

3542-Pos Board B403
Dynamics of A-Lattice Microtubules
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Microtubules are intrinsically dynamic structures whose polymerisation is subject to extensive spatial and temporal control in cells, partly through the activity of microtubule-associated proteins. Microtubules can potentially assemble with two different lattice arrangements of heterodimers. Lateral contacts of heterodimer subunits may be either alpha-beta, making an A-lattice, or alpha-alpha and beta-beta, forming a B-lattice. 13-protofilament B-lattice microtubules contain a single seam of A-lattice contacts. A-lattice microtubules are composed purely of these seams. Since microtubules assembled in vitro have predominantly the B-lattice arrangement, it has not previously been possible to study the dynamics of the A-lattice. We recently found that Mal3, the EB1-homologue in *S. pombe*, stabilises the A-lattice and promotes assembly of 13-protofilament A-lattice-containing microtubules. We are now analysing the dynamics of Mal3-induced A-lattice microtubules in vitro. We measured the gliding velocity and the shrinkage rate of GMPCPP stabilised A-lattice and B-lattice microtubules during gliding on a rat kinesin-1 coated-glass surface in the absence of free tubulin. The gliding velocity of A-lattice microtubules was similar to that of B-lattice microtubules. On the other hand, the plus end of A-lattice microtubules shrank 20 times faster than the plus end of B-lattice microtubules, and 10 times faster than the minus end of A-lattice microtubules. This suggests that tubulin heterodimer subunits dissociate faster from the A-lattice than from the B-lattice and that the A-lattice is therefore less stable than the B-lattice. The A-lattice seam in B-lattice microtubules may therefore be a zone of unusual structural weakness that would provide an opportunity for regulating microtubule dynamics via MAPs that suppress the dissociation of subunits from the A-lattice.

3543-Pos Board B404
Characterization of Tubulin Derived from *Ginkgo Biloba* and its Interaction with *Rice* Plant Kinesin
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Previously, we have expressed novel *Rice* plant specific kinesins and studied their biochemical characterization. The plant kinesins showed very unique properties. Especially ATPase activities of the kinesins were relatively much lower than that of conventional kinesin. Moreover, the kinesins did not show motile activity on the microtubules prepared from porcine brain. Although the structure of tubulin is well conserved, it is demonstrated that the plant tubulin has different characteristics from tubulin derived from animal. Therefore, plant kinesin may be more compatible with plant tubulin than animal tubulin. In this study, we prepared the plant tubulin from the pollen of *Ginkgo biloba*. Native tubulin was prepared from ground pollen by acetone treatment methods and purified with DEAE, Mono Q column and Gel filtration chromatography. We have also cloned cDNA of the α/β tubulin from the *Ginkgo biloba* leaves by RT-PCR. The recombinant α/β tubulins were expressed by *E. coli*. bacterial expression system. The recombinant tubulins were purified by Co^{2+} chelating column.
Polymerization of the purified plant tubulin to microtubule was monitored by measuring the increase of absorption at 350 nm. Negative staining electron microscopic analysis also revealed the microtubule configuration. ATPase activity of the *Rice* plant kinesin, K16 was activated by plant tubulin more significantly than that of animal tubulin. These results suggested that plant kinesin is more compatible with plant kinesins than kinesin derived from animal.

3544-Pos Board B405
Alp7 Potentiates Microtubule Tip Tracking by the Processive Plus End Polymerase Alp14
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TOG-family polymerases track microtubule plus ends in vivo and modulate microtubule dynamics. Several different model mechanisms for the molecular mechanism of action of these proteins have been proposed (Kerssemakers et al. (2006) Nature 442:709; Al-Bassam et al (2006) JCB 172:1009; Brouhard et al. (2008) Cell 132:79). To test candidate models in more detail, we have expressed and purified Alp14 and Alp7 using a baculovirus system and reconstituted their activities in vitro using dynamically unstable microtubules built from purified single isoform (a1b) S.pombe tubulin. Alp14 deletion mutants show an in vivo defect in microtubule assembly (Sato et al. (2004) MCB 15:1609), consistent with an effect on microtubule dynamics. We find that in vitro, Alp14 accelerates the shrinkage of GMPCPP microtubules 2x and the growth of GTP-microtubules up to 10x. Changes in growth rate within a growth phase correspond to changes in the amount of Alp14 at the tip. The acceleration of growth by Alp14 causes a decrease in catastrophe frequency consistent with extension of the GTP-cap. Remarkably, although Alp14 binds to mammalian brain tubulin, it does not accelerate the growth of mammalian brain microtubules. Instead, Alp14 is competitively inhibited by mammalian brain tubulin. Tip tracking by Alp14 is tightly linked to the catalysis of microtubule growth: Alp14 loses its tip tracking ability upon the addition of 10% mammalian brain tubulin. The addition of the TACC-protein Alp7 restores the tip-tracking ability of Alp14, but not its ability to enhance the microtubule growth rate. This result is consistent with reports that Alp7 is a localization factor of Alp14 in vivo (Sato et al. (2004) MCB 15:1609). On dynamic S.pombe microtubules, Alp7 enhances the processivity of Alp14, causing sustained fast growth and correspondingly reduced catastrophe.

3545-Pos Board B406
Microtubule Dynamics of S. Pombe Tubulin
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We have developed a fission yeast system for expression and purification of single isoform tubulin. Replacing the non-essential *S. pombe* α2 tubulin gene with a second copy of the α1 encoding gene creates a strain expressing single α1 and β tubulin isoforms. We obtain yields of about 10 mg of highly purified (>99%) single isoform tubulin from an 80 l culture. Mass spectroscopy of the purified tubulin detects no post-translational modifications. Dynamic microtubules were nucleated from axonemes, recorded by video enhanced DIC microscopy and microtubule lengths measured. Microtubule growth rates increase linearly with tubulin concentration at both the fast and slow growing microtubule ends. Rate constants were determined using a simple bimolecular model. α1β tubulin has fast end k_{on} 5.5 uM^{-1} s^{-1} and k_{off} 6.7 s^{-1}, and slow end k_{on} 3.1 uM^{-1} s^{-1} and k_{off} 2.8 s^{-1}. Mixed isoform α1α2β has fast end k_{on} 7.6 uM s^{-1} and k_{off} 15.1 s^{-1} and slow end k_{on} 2.6 uM^{-1} s^{-1} and k_{off} 5.0 s^{-1}. The Kd for both microtubule ends was ~2uM for α1α2β and ~1.1 uM for α1β suggesting similar binding affinities for tubulin heterodimers at fast and slow ends, but different kinetics. Following catastrophe the rate of rapid shrinkage was independent of the free tubulin concentration and about 2x faster at the fast compared to the slow ends: 218 ± 16 s^{-1} compared to 124 ± 23 s^{-1} for α1α2β and 263 ± 26 s^{-1} compared to 111 ± 29 s^{-1} for α1β. We conclude that *S. pombe* microtubule dynamics are qualitatively similar to those of brain tubulin microtubules but the kinetic rates are different, consistent with *S. pombe* tubulin assembling at lower concentrations than brain tubulin. We are now comparing *S. pombe* microtubule growth with that of brain tubulin at higher resolution.

3546-Pos Board B407
Simultaneous Measurement of Microtubule Protofilament Number and Bending Stiffness
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Microtubules are nanometer-scale cytoskeletal polymers which play a role in cell division, cell structure, and intracellular transport. Each of these functions requires microtubules that are stiff and straight enough to span a significant fraction of the cell diameter. Moreover, microtubules come in a variety of structures characterized by different diameters, or equivalently, different numbers of

protofilaments. Previous experiments to characterize the stiffness of microtubules have generally relied on heterogeneous ensembles of microtubules with different structures, complicating the interpretation and comparison of stiffness measurements. We report on a kinesin-driven microtubule gliding experiment that simultaneously determines the stiffness and protofilament number (diameter) of individual microtubules, allowing us to distinguish stiffness for microtubules of 12-14 protofilaments (including two different 14 protofilament lattice structures). The scaling of our stiffness measurements are consistent with previous theories connecting protofilament number to stiffness, but also suggest an alternate correlation between stiffness and protofilament superhelical pitch.

3547-Pos Board B408

A Structure-Function Study of Map Tau: Analyzing Distinct Map Tau Domains in Mediating Microtubule Assembly and Bundling using Synchrotron SAXS

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The microtubule-associated protein tau (MAP tau) promotes the assembly of tubulin into microtubules, a major component of the eukaryotic cytoskeleton. In neurons, MAP tau is especially abundant in the axon and plays a critical role in axonogenesis [1]. However, aberrant tau function has been implicated in many neurodegenerative diseases, such as Alzheimer's and supranuclear palsy [2,3]. Thus, there is a clear need to understand the structure of MAP tau with regards to its interactions with microtubules and its role in neuropathies. The six isoforms of MAP tau are often described as sequentially having a N-terminal (consisting of a projection domain and a proline-rich region), microtubule-binding repeats, and a C-terminal. While the function of the microtubule-binding repeats is well understood, the N- and C- terminals have been particularly difficult to characterize structurally and functionally due to the presence of intrinsically disordered domains. Using synchrotron small-angle X-ray scattering (SAXS), we examined the higher-order assembly of microtubules induced by varying concentrations of wild-type MAP tau under cell-free solution conditions. The functional dependence of the projection-domain, proline-rich region, and C-terminal were also examined by utilizing distinct constructs resulting in MAP tau with deletion domains. Not only do these results correspond well to axonal microtubule-tau bundles in vivo, we find that the modulation of the bundling of MAP tau is isoform-dependent. Surprisingly, the N-terminal tail of MAP tau is not necessary for higher-order assembly, as some models have suggested [4].

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3548-Pos Board B409

Stu2p, the Budding Yeast Homologue of XMAP215, is a Weak Microtubule Polymerase that Promotes Rescues

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Stu2p is a member of the Dis1/XMAP215 family of polymerases and is required for spindle orientation and metaphase chromosome alignment, and for anaphase B spindle elongation. Despite Stu2p having a phenotype suggesting that it promotes microtubule growth, studies till now with purified proteins indicate only that Stu2p antagonizes growth. To investigate the effect of Stu2p on microtubule dynamics in vitro, we purified recombinant Stu2p from insect cells and studied its effect in a total-internal-reflection fluorescence (TIRF) microscopy assay: GMPCPP-stabilized microtubules (seeds) were immobilized on the glass surface and varying amounts of fluorescently labeled porcine-brain tubulin, GTP and Stu2p proteins were added to the chamber. Images were acquired using time-lapse microscopy. In the presence of tubulin, Stu2p increased the microtubule growth rate, though to a lesser extent than XMAP215. Stu2p had no effect on the catastrophe frequency, but significantly increased the rescue frequency. In conclusion, Stu2p shares functional properties with its metazoan homolog XMAP215, though its polymerase activity is weaker. These properties go some way to explaining the phenotypes of the Stu2p-depleted cells.

3549-Pos Board B410

Cooperative Interactions in the Microtubule-Severing AAA ATPase Spastin

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Spastin is a hexameric ring AAA ATPase that severs microtubules. To see how the ring complex funnels the energy of one or multiple ATP hydrolysis events to the site of mechanical action, we investigate here the cooperativity of spastin. Several lines of evidence show that two neighbor subunits interact strongly with each other: (i) the ATP-activated ATPase activity shows a Hill coefficient of ~2; (ii) inactive mutant subunits and (iii) non-hydrolyzable ATP analogs inhibit the activity of spastin in a hyperbolic dependence, characteristic for two interacting species. A quantitative model fits the data well, and strongly disfavors orders of cooperativity higher than 2. These observations are relevant for patients suffering from SPG4-type hereditary spastic paraplegia, and can explain why single amino acid exchanges lead to dominant-negative phenotypes. In severing assays, wildtype spastin is even more sensitive towards the presence inactive mutants than in enzymatic assays, suggesting a weak coupling of ATPase and severing activity. Together, these observations indicate that each of spastin's six catalytic sites depends on the presence of an active neighbor site, and that ATP hydrolysis in all subunits is required for full severing activity.

3550-Pos Board B411

One-Dimensional Diffusion of Tau Protein Guided by the Microtubule Lattice

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Current models for the intracellular transport of Tau protein suggest motor protein dependent co-transport with short microtubule fragments and diffusion of Tau in the cytoplasm. In these models Tau is believed to be stationary bound to microtubules and in equilibrium with free diffusion in the cytosol. Recent observations that some members of the microtubule-dependent kinesin family show Brownian motion along MTs led us to hypothesize that diffusion along MTs could also be relevant in the case of Tau. We used single molecule TIRF microscopy to probe for diffusion of individual fluorescently labelled Tau molecules along immobilized microtubules. This allowed us to circumvent masking of microtubules dependent diffusion of Tau by excess of labelled Tau protein in solution that might occur in in vivo overexpression experiments. We found that about half of the individually detected Tau molecules were able to diffuse bi-directionally along microtubules in the absence of ATP. Diffusion parameters such as diffusion coefficient, interaction time, and scanned microtubule length did not change with Tau concentration. Tau binding and diffusion of Tau along the microtubule lattice were sensitive to ionic strength and drastically reduced upon enzymatic removal of the negatively charged carboxy termini of tubulin. We propose one-dimensional Tau diffusion guided by the microtubule lattice as one possible additional mechanism for the distribution of Tau. By such one-dimensional microtubule lattice diffusion instead of or in addition to directed motor-dependent transport, both ends of the microtubules, i.e. the sites where Tau protein is needed during microtubule polymerisation, could be reached even in situations when Tau levels are pathologically high and active transport along microtubules might be compromised.

3551-Pos Board B412

Biophysical Study of Native Yeast Kinetochores Indicates Distinct Roles for Phospho-Regulation of Core Microtubule-Binding Subcomplexes

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Chromosome segregation is orchestrated by multi-protein complexes called kinetochores, which initially bind microtubules and later form persistent load-bearing tip attachments. Accurate segregation requires regulation of kinetochores by Aurora B kinase. One popular view is that Aurora acts on kinetochores that have already established erroneous attachments to microtubule tips, phosphorylating two major microtubule-binding subcomplexes, Ndc80 and Dam1, and thereby triggering detachment. It is unknown whether phosphorylation affects initial binding of kinetochores to microtubules. Moreover, the relative importance of phosphoregulation of these subcomplexes in the context of whole kinetochores is unclear. To address these